Relatedness and body size influence territorial behaviour in *Salmo salar* juveniles in the wild

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Running headline: Territorial behaviour in *Salmo salar*

Abstract
Wild Atlantic salmon, *Salmo salar* L., juveniles defend territories to enable exclusive access to food resources, and kin selection benefits may be accrued where territorial boundaries of relatives overlap. This study explored space sharing events between pairs of sibling and non-sibling fish as a measure of territoriality and resource competition in a small chalk stream using passive integrated transponder (PIT) technology. The time period between fish detections in a shared space was closer between pairs of siblings (sibling pairs mean = 60.48 ± 51.84 min; non-sibling pairs mean = 348.8 ± 65.94 min). These results suggest that the territorial boundaries of related fish often overlap, thus increasing the likelihood of siblings accruing kin selection benefits. The findings from this study also suggest that outcomes of competitive interactions among dominant and subordinate fish are less pronounced when fish are related.

Keywords
Atlantic salmon, competition, dominance, kin selection, kinship, tagging

Introduction

Many animal species are territorial, defending a portion of their home range to the exclusion of other individuals. Territoriality is common among birds (Viera, Viblanc, Filippi-Codaccioni, Côté & Groscolas, 2011), reptiles (Manteuffel & Eiblmaier, 2008), insects (Tanner & Adler, 2009) and fish (Tricas, 1989). Variation in habitat suitability may act to limit dispersal. On spawning, adult female Atlantic salmon, *Salmo salar* L., deposit their eggs into a series of nests excavated in the river substrate which, when aggregated, form a redd (Fleming, 1998). The subsequent synchronous nocturnal emergence and dispersal of fry from a redd is a predator avoidance tactic (Riley & Moore, 2000), with initial dispersal distances limited to tens of metres downstream (Crisp, 1995). Large numbers of siblings dispersing into spatially limited locations could make kin-biased behaviour important as the juvenile salmon subsequently establish and defend territories against intruders in an attempt to gain access to a limited food supply (Brown & Brown, 1992; Brown & Brown, 1993a). These territories interconnect to form a mosaic across the streambed (Grant, Steingrímsson, Kelley & Cunjack, 1998), and it may be possible that kin-biased behaviour at these boundaries plays a pivotal role in moderating territorial disputes among fish (Brown & Brown, 1993b; Brown & Brown, 1996a; Quinn, Dittman, Peterson & Volk, 1994). For example, previous work has shown that competition among individuals may be lower among genetically diverse groups (e.g. non-kin groups) than genetically similar groups (e.g. kin groups) owing to feeding specialisms and thus differing habitat preferences by genetically distinct components of the population (Griffiths & Armstrong, 2001; Fernandes, Copp & Riley, 2016). However, there is also evidence to suggest that relatives reduce aggressive behaviour towards one another and thus maximise kin selection benefits by association (Griffiths & Armstrong, 2002). It seems likely, therefore, that the cost-benefit trade-off associated with kin-biased behaviour is dependent on specific ecological conditions (Fernandes, Griffiths, Ibbotson, Bruford & Riley, 2015), life history stage (Fernandes, Ibbotson *et al*., 2015), relative dominance status and the opportunity to interact with conspecifics.

In the wild, food distribution varies considerably, and dominant juvenile salmon are more likely to monopolise favourable feeding areas with a stable food supply (Martin-Smith & Armstrong, 2002), to achieve higher growth rates (Metcalfe, Huntingford, Thorpe & Adams, 1990). A difference in body size as small as 5% can result in larger individuals dominating over smaller individuals (Abbott, Dunbrack & Orr, 1985). When territorial neighbours are similar in size, total aggression levels among a pair of fish is highest when each fish has equal ability to defend a resource (Getty, 1989). However, territories are not

fixed in space and the ranges of several fish may overlap (Armstrong, Huntingford & Herbert, 1999), therefore there is the potential for kin-biased behaviour to occur where relatives' territories connect or overlie. Dominant fish being more tolerant of subordinates when they are related than unrelated (Brown & Brown, 1996b) begs the question of whether territory sharing occurs more often among siblings than non-siblings.

In this study, passive integrated transponder (PIT) technology was used to study territorial behaviour between pairs of sibling and non-sibling juvenile Atlantic salmon (*Salmo salar* L.) sharing a space in the wild. Investigations were made on the frequency of space sharing events between pairs of siblings and pairs of non-siblings; the mean time intervals between fish displacing one another in a shared space, and whether this was influenced by relatedness; and the proportion of time the larger dominant fish (dependent on the length difference between pairs of fish at tagging) occupied a territory (space occupancy) shared between pairs of siblings and between pairs of non-siblings.

Methods

Juvenile *Salmo salar* were reared from wild parents, from which a tissue sample (adipose fin clip) was taken for genetic analysis, to create distinct full-sibling family groups in two consecutive years (2006 & 2007). Three family groups were chosen for use in each year based on their similarities for length (24.8–27.2 mm FL), weight (0.126–0.177 g) and emergence time, as part of a programme investigating the effect of kinship and family traits on growth rate (Fernandes, Griffiths *et al*., 2015), migration strategies (Fernandes *et al*., 2015) and microhabitat use (Fernandes *et al*., 2016).

In each year, *S. salar* fry were stocked in late March and early April in a 1.5-km stretch of the River Cerne a tributary of the River Frome, Dorset, UK, into designated full-sibling and mixed-sibling (formed by combining equal numbers of fish from the full-sibling family groups) sites. In year 1, fry were stocked at a density of 2.7 fish/m2 into six full-sibling and six mixed-sibling sites, each 30 m in length, alternated along the river, with gaps between stocked sites of 100 m. In year 2, fry were stocked at a density of 4.1 fish/m2 into three full-sibling sites, each 50 m in length and one mixed-sibling site, of 150 m in length, located furthest downstream, with the gaps between each stocking site increased to 250 m. Fish were given time to establish territories and grow before the sites were sampled by electric fishing during the corresponding summers. At this time, all juvenile *S. salar* parr caught were tagged with passive integrated transponder (PIT) tags as per Riley, Eagle, Ives, Rycroft and Wilkinson (2003), and weight and length measurements were noted. A tissue

sample (adipose fin clip) was also taken to assign all parr to their family group by genetic analysis. Fish were released back to their location of capture following recovery from anaesthesia (See Fernandes, Griffiths *et al*., 2015 for full details of experimental design).

To determine parr space sharing, each year portable PIT multipoint decoder MPD units (Riley *et al*., 2003) were installed at one full-sibling and one mixed-sibling stocked area simultaneously, generating data over a 4-day period (a "replicate"). Each PIT-tag detection may be considered as a "point sample" (i.e. the fixed occurrence of an individual fish in time and space; c.f. Riley *et al*., 2003). (See Fernandes *et al*., 2016 for full details of field data collection).

Raw data were gleaned and parsed, which revealed three emergent categories of space sharing (levels). Data were categorised by the number of sequential detections between a pair of fish on the MPD antenna: two sequential detections = low space sharing; three sequential detections = moderate space sharing; and four or more sequential detections = high space sharing. The strength of the space sharing level may be an indicator of habitat quality, with low space sharing being habitats where resources are low, and high space sharing being prevalent in the most favourable habitats. Encounter events (sequential detections) between fish in one category were not included in other categories to reduce bias and pseudoreplication in the experimental design. Following initial data analysis, multiple events of space sharing were found taking place between the same pairs of fish, therefore to limit pseudoreplication, only the first event between pairs of fish on each antenna were included in the final data set. Some individuals appeared more than once (in more than one event between different fish), hence each fish was assigned an identification number which was set as a random factor in the analyses. To investigate whether the time frame between one fish displacing another on an MPD antenna decreased over the duration of a space sharing event owing to a development of familiarity, "order of time intervals" was incorporated into the model.

To test whether the time intervals between two sequential fish being detected on the same MPD antenna was influenced by relatedness, a GLMM was carried out in ASReml v.2.0. Each space sharing category was analysed separately. The dependent term in the model was "time intervals" (covariate) in seconds. The main terms and interactions between terms in the starting model were sibship (sibling or non-sibling; Factor), order of time intervals (Factor), sibship x order of time intervals. The identity of individual fish was set as a random effect to account for data collected repeatedly from the same individual. At the low space

sharing level, the small sample size meant that only the factor "sibship" could be accounted for by the GLMM model.

To test the effect of body size (length) difference on the space occupancy of larger fish "space occupancy" (the number of detections at one MPD antenna as a percentage of the total number of detections between a pair fish sharing space) was calculated for pairs of siblings and non-siblings at all space sharing levels. The difference in fork length (cm; previously measured at the time of PIT tagging) between fish at each space sharing event was calculated to test whether the relative size of the fish influenced the proportion of detections on an antenna. All proportion data were arcsine transformed to achieve normality. Only the data describing the largest of the pair of fish (assumed to be the dominant fish) were used in the analysis. Each space sharing category was analysed separately so that comparisons could be made between sibship effects at each level. The dependent term in the model was "space occupancy" (arcsine transformed proportion of time spent on an antenna). The main terms and interactions between terms in the starting model were sibship (sibling or non-sibling; Factor), difference in length (Covariate), sibship x difference in length. The identity of individual fish was set as a random effect to account for data collected repeatedly from the same individual.

Results

Fish from all six family groups were detected by the PIT MPD units. As indicated by the number of MPD detections, fish from family 4 was the most abundant full-sibling group (n = 28 fish), followed by family 3 (n = 9 fish), and family 6 (n = 9 fish), family 2 (n = 5 fish), family 1 (n = 4 fish) and family 5 (n = 2 fish). There was no significant effect of individual fish (random effect) on time intervals and on the proportion of time spent on an MPD antenna (at all space sharing levels; $P \geq .05$). There was no significant relationship between the density (total number of fish) found within a stream site (approximately 2 weeks prior to the installation of the MPD units) and the number of space sharing events (including observations on pairs of fish more than once in a space sharing level) at all levels (Spearman's rank correlation (two-tailed); low P = .55, moderate P = .30 and high P = .33). The observed ratio (number of events amongst pairs of siblings to pairs of non-siblings) at all space sharing levels for each MPD replicate did not deviate significantly (G test, $P > .05$) from the expected ratio (calculated from the number of fish from each family group detected for each MPD replicate (n = 31). Sites stocked as full-siblings contributed to the data set 15 interactions between full-siblings, and one interaction between non-siblings, whereas sites

stocked as mixed-sibling sites contributed to the data set six interactions between full-siblings, and nine interactions between non-siblings.

Encounters between full-sibling pairs were more frequent largely due to the stocking strategy, which was designed to accommodate several simultaneous investigations on juvenile *S. salar* kinship. Nevertheless, the time intervals between sequential detections were shorter for pairs of siblings (sibling pairs mean = 60.48 min ± 51.84, non-sibling pairs mean = 348.8 min ± 65.94), significant at the high space sharing level ($F_{1,57.0} = 11.76$, $P = .001$). With the proportion of time spent in a space; as detected by an MPD antennae (by the largest fish of pair), significantly influenced by the interaction between sibship and relative fork length at each level (Figure 1a–c), although the relationship varied between space sharing levels as follows: At the low space sharing level, the time spent on an MPD antenna (by the largest fish) significantly increased with increasing difference in fork length between the fish pair ($F_{1,2.0} = 163.52$, $P = .006$, Figure 1a). At the moderate space sharing level, the interaction between sibship and length difference significantly affected proportion of time spent on an MPD antenna; length difference between pairs of siblings showed little effect on the proportion of time spent on an MPD antenna, but non-sibling pairs showed a negative correlation ($F_{1,5.0} = 6.57$, $P = .050$, Figure 1b). At the high space sharing level, length difference had a significant negative effect on proportion of time spent on an MPD antenna ($F_{1,7.0} = 11.14$, $P = .012$, Figure 1c) with closely size-matched fish spending more time on antennae. The number of detections (log10 transformed) did not change significantly (Kruskal–Wallis test, $P = .787$) over time (n = 4 days) following the installation of the MPD units in each replicate site (n = 12), suggesting minimal disturbance to fish behaviour.

Discussion

The present study supports the hypothesis that sibling *S. salar* parr in the wild may bias their behaviour towards relatives when a space is shared through territory overlap, thereby enabling the possibility of accruing kin selection benefits. The findings suggest that sibship was influential in the sharing of space, resulting in significantly shorter interdetection time intervals occurring between pairs of siblings in the high space sharing category. Furthermore, the findings suggest that, under the natural ecological conditions prevalent during this experiment (see Fernandes, Griffiths *et al*., 2015), the advantages of kin-biased behaviour are shared more equally between dominant and subordinate siblings through more equitable allocation of time spent occupying shared space. Previous work by Griffiths and Armstrong (2002) showed that subordinate fish tested in a naturalistic indoor stream gain twofold

foraging advantages during close association with dominant siblings owing to reduced agonistic behaviour and increased territory sharing. However, the direct and indirect fitness benefits afforded via changes in kin-biased behaviour may vary between habitats with changes in territory quality (Brown & Brown, 1993b).

Previous genetic studies have failed to find evidence of *S. salar* sibling aggregation in the wild (see Fontaine & Dodson, 1999; Garant, Dodson & Bernatchez, 2000; Olsén, Petersson, Ragnarsson, Lundqvist & Järvi, 2004; Brodeur *et al*., 2008), despite the advantages of associating with relatives implicit from laboratory studies. Many genetic studies, however, have calculated relatedness of fishes caught together in relatively long stream stretches varying from 50 to 300 m (Carlsson, Olsén, Nilsson, Overli & Stabell, 1999; Mjølnerød, Refseth & Hindar, 1999; Garant *et al*., 2000). However, in the present study, patterns of association in short river sections were investigated and analysed with respect to the strength and time interval sequential detection of pairs of fish on the same MPD antenna, thus providing a level of detail absent from most other studies (e.g. Carlsson *et al*., 1999; Mjølnerød *et al*., 1999; Garant *et al*., 2000).

The size of an individual's territory is limited by food abundance and competitive pressure from conspecifics (Valdimarsson & Metcalfe, 2001). In the present study, the proportion of time spent on an MPD antenna was influenced by the fork length difference at both the low and high space sharing level. At the low space sharing level dominant fish spent a significantly higher proportion of time dominating the area, whereas the opposite trend was revealed at the high space sharing level. Perhaps in areas on the streambed where habitat quality is high, territory sizes might be smaller and territorial aggression may be limited owing to high food abundance. Conversely, where habitat quality is low, it may benefit individuals to have larger territory sizes to increase feeding opportunities (Brown & Brown, 1993b), and thus driving the defence at territory boundaries.

Dominant fish can benefit from the presence of subordinates because their presence allows the burden of territory defence to be shared and thus foraging efficiency to increase (Clifton, 1990). Intriguingly, at the moderate space sharing level, the present study shows that sibling pairs of dominant and subordinate fish were equally competitive for space occupancy, and dominant fish spent less time in shared spaces with unrelated subordinates. Despite the body size difference, the dominant fish did not monopolise a shared space when in pairs with siblings (see also Brown & Brown, 1993b; Brown & Brown, 1996a) and this may suggest that under some circumstances subordinate fish in wild populations gain twofold foraging advantages by close association with dominant siblings (Griffiths & Armstrong, 2002). Kin

selection benefits are not necessarily dependent upon territories bordering those of siblings, but can be accrued during occasional associations by way of overlapping home ranges (Griffiths & Armstrong, 2002). However if resource availability is low, the cost of helping a relative rises and individuals may be less willing to pay the cost of helping, as this may result in decreased personal access to a limited resource (Fernandes, Griffiths *et al*., 2015). Therefore, to maximise smolt output from salmon stocking programmes, fisheries managers could consider releasing high densities of closely related individuals in highly productive stream sections where the benefits of kin-selection are predicted to be greatest, and lower densities of unrelated individuals in less productive, or highly diverse, stream sections where microhabitat breadth influenced by the family of origin may reduce intraspecific competition (Fernandes *et al*., 2016).

The findings from the present study are based on relatively low numbers of fish detections, and therefore the results may not be representative of the *S. salar* parr territorial sharing and overlap occurring for the whole population. Nonetheless, this study has provided preliminary findings that will be useful when designing future large scale studies, which provide fisheries managers with an insight into the importance of the interaction between the environment and family traits and the role these play in mediating space sharing events, space occupancy and regulating densities in territorial fish.

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